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RADIOIODIDE IN THE THYROID AND IN OTHER ORGANS OF RATS TREATED WITH LARGE DOSES OF PERCHLORATE[1]

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WE INVESTIGATED the hitherto largely unexplored first step of thyroid hormone biogenesis, the diffusion of iodide into the thyroid, by administering radioiodide after complete blocking of the thyroidal iodide pump ("trap") with its most potent inhibitor, sodium perchlorate (Wyngaarden *et al.*, 1952). We correlated the amount of radioiodide that was found in the thyroid under such conditions with the structure of the gland, which was experimentally altered by varying the intensity of thyrotrophic stimulation. It was our hope that such a study may yield information concerning a) the site of the iodide "trap" within the thyroid parenchyma and b) the possible influence of thyrotrophin on the permeability of the thyroid cell to iodide. Levine and Goldstein (1955) have recently emphasized, in discussing the mode of action of insulin, that hormones may regulate metabolic processes by altering the permeability of cell membranes to specific substances.

Our investigations were then extended to the effect of perchlorate on a) the distribution of radioiodide in organs other than the thyroid, b) the radioiodide space of the whole body, c) the gastric iodide pump, d) intestinal absorption of radioiodide and e) excretion of radioiodide.

MATERIALS AND METHODS

1. *Experimental animals*

This study was performed on young adult male rats of the Sprague-Dawley strain which were maintained on Rockland pellets and tap water.

2. *Injections*

Injections were given subcutaneously.

a) *Propylthiouracil (PTU).* All animals received PTU to prevent organic binding of radioiodine. Unless otherwise specified, a single injection of 6 mg. of PTU in solution form was given.

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b) *Perchlorate.* Experiments on rats with highly active thyroidal iodide "traps," the results of which are included in Table 1, showed that 100 mg. of $NaClO_4$ was as effective as 200 or 400 mg. in abolishing the function of the iodide concentrating mechanism. Results obtained with these three dosages were therefore pooled in preparing Table 1, and in subsequent experiments 100 mg. of $NaClO_4$ was used.

c) *Chloride.* NaCl was given in a dose (47.5 mg.) equal to 100 mg. of $NaClO_4$ on a molar basis to rats which served as controls to the perchlorate-treated animals.

d) *Stable iodide.* When NaI was administered, the dosage (122 mg.) was also the molar equivalent of 100 mg. of $NaClO_4$. In one experiment (Table 1) 100 mg. of NaI was administered.

e) *Treatments designed to alter thyroid structure* are described in detail in the footnotes to Table 1. PTU was given together with TSH since it is known to enhance the effectiveness of this hormone (Halmi and Spirtos, 1954). Prolonged treatment with PTU was instituted to produce goiter. It was followed by three triiodothyronine injections in order to depress the activity of the stimulated iodide "trap" without abolishing the goiter. This was considered necessary because we assumed that only toxic or lethal doses of perchlorate may block the thyroidal iodide "trap" completely if the thyroid-serum radioiodide gradient is very high, as it is likely to be after chronic PTU administration. Four-hundred mg. of $NaClO_4$ was found to be close to the LD_{50}.

f) *Radioiodide.* Carrier-free I^{131} was given in doses ranging from 5 to 50 μc.

g) *Schedules.* When the rats were killed 1–1½ hours after the administration of radioiodide, the schedule of injections was as follows:

0 minute : 6 mg. PTU + 50 mg. $NaClO_4$[5] or 23.7 mg. NaCl or 61 mg. NaI
45 minutes: I^{131} + 50 mg. $NaClO_4$ or 23.8 mg. NaCl or 61 mg. NaI
103–135 minutes: Sacrifice

When the rats were sacrificed 4–4½ hours after radioiodide administration, the schedule was the following:

0 minute : 6 mg. PTU + 50 mg. $NaClO_4$ or 23.7 mg. NaCl or 61 mg. NaI
45 minutes: I^{131} + 25 mg. $NaClO_4$ or 11.9 mg. NaCl or 30.5 mg. NaI
165 minutes: 25 mg. $NaClO_4$ or 11.9 mg. NaCl or 30.5 mg. NaI
285–315 minutes: Sacrifice.

In experiment XVI the 24 rats received a total of 52 mg. of PTU according to the following schedule:

0 hour : 20 mg. PTU suspension
24 hours: 6 mg. PTU solution
32 hours: 20 mg. PTU suspension
44 hours: 6 mg. PTU solution
48–48½ hours: Sacrifice

Sixteen rats were given the radioiodide at 24 hours and 8 rats at 47 hours. Eight rats of the former group and all the animals of the latter received 47.5 mg. of NaCl at 47 hours. The other 8 rats of the first group were injected with 100 mg. of $NaClO_4$ at 47 hours.

3. *Operative procedures*

a) *Nephrectomy.* Bilateral nephrectomy was performed through a single dorsal incision. The rats were anesthetized with nembutal. In experiment XV nephrectomy was carried out transperitoneally since it was combined with ligation of the cardia and the pylorus.

[5] When a total dose of 200 or 400 mg. of $NaClO_4$ was given, the single doses were proportionately higher.

TABLE 1. THYROID:BLOOD RADIOIODIDE CONCENTRATION (T/B) RATIOS, AS AFFECTED BY PERCHLORATE. CORRELATION WITH THYROID STRUCTURE

Group	Iodide administered	Hours after I^{131}	T/B ratio		% of total gland volume			Gland weight, mg.
			Without ClO_4^-	With ClO_4^- [1]	I^{131} Space	Stroma	Stroma + cells	
Intact	tracer I^{131}	1-1½	(6) 22.7 ± 1.9[5]	(12) 0.45 ± 0.025	30	(10) 10.2 ± 0.72	85.2 ± 0.45	(18) 8.3 ± 0.35
Hypex[2]	tracer I^{131}	1-1½	(6) 2.5 ± 0.72	(16) 0.47 ± 0.018	31	(10) 8.6 ± 0.53	62.0 ± 2.3	(22) 8.2 ± 0.33
Hypex+PTU+TSH[3]	tracer I^{131}	1-1½	(8) 83.6 ± 9.2	(20) 0.44 ± 0.022	29	(10) 10.4 ± 0.72	75.4 ± 1.7	(28) 8.1 ± 0.27
PTU+triiodo-[4] thyronine	tracer I^{131}	1-1½	(6) 56.5 ± 4.4	(14) 0.43 ± 0.015	29	(10) 12.1 ± 0.46	83.7 ± 1.3	(20) 22.1 ± 1.1
Intact	tracer I^{131}	4-4½		(11) 0.41 ± 0.029	28		--	(11) 7.9 ± 0.25
Intact	tracer I^{131} 45 min. after 100 mg. of NaI^{127}	1-1½	--	(8) 0.44 ± 0.017	29	--	--	(8) 9.7 ± 1.1

[1] In parentheses: number of rats in group used for determinations.
[2] 100-400 mg. $NaClO_4$.
[3] Nine days after hypophysectomy.
[4] Nine days after hypophysectomy. Twenty mg. of propylthiouracil in suspension form and 0.9 U.S.P. unit of TSH injected 24 hours before I^{131} administration.
[5] Twenty mg. of propylthiouracil in suspension form injected daily for 14 days; thereafter 30 μg. of l-triiodothyronine daily for 3 days. Animals killed 24 hours after the last injection.
[6] Mean ± standard error.

b) *Cardiac and pyloric ligation* was performed under nembutal anesthesia in such rats as were subsequently used for the determination of gastric juice-serum radioiodide gradients. The operation was followed immediately by the injection of PTU (see schedules).

c) *Intestinal ligation and radioiodide injection.* In order to study the absorption of radioiodide from the small intestine, the following procedure was carried out. Rats which had received 100 mg. of $NaClO_4$ or 47.5 mg. of NaCl one hour earlier were laparotomized under nembutal anesthesia and ligatures were applied to the pylorus and the cecal end of the ileum. Radioiodide was injected into the duodenum through a gauge 27 hypodermic needle. Thereafter the intestine was placed back into the abdominal cavity and the skin incision was closed with surgical clips. When the rats were sacrificed 30 minutes later, the small intestine was removed *in toto* and dissected free of its mesentery.

4. *Methods used in securing samples of body fluids and tissues and in determining their radioactivity*

Blood was obtained from the abdominal aorta. Some organ-serum radioiodide concentration (O/S) ratios were determined by the method of VanderLaan and Greer (1950). Tissue samples not weighing over 50 mg. were squashed on filter paper under Scotch tape and 0.1 ml. of serum or a 1:10 dilution thereof was pipetted onto filter paper, dried and covered with Scotch tape. The radioactivity of the tissue and serum samples was determined by means of a thin mica end-window Geiger-Müller (beta) counter. The O/S ratio was expressed as the quotient of the activity of 100 mg. of wet tissue over that of 0.1 ml. of serum. This method was used for thyroid, salivary glands, pituitary, adrenals and diaphragm. For thyroid-blood radioiodide concentration ratio determinations 0.1 ml. of blood was laked in 0.9 ml. of distilled water and 0.1 ml. of the hemolysate was used in the manner outlined for serum. In the case of larger organs (kidney, lung, skin, liver, testis, gastrocnemius muscle and stomach wall) either the whole organ or a piece not weighing over 2 gm. was put into plastic tubes, serum samples were collected in similar tubes, radioactivities were determined using a well-type gamma counter (Texas Company), and O/S ratios were computed. Measured stomach juice samples were obtained from the supernatant of centrifuged gastric contents and gamma-counted in plastic tubes. Erythrocyte-plasma radioiodide concentration ratios were determined by collecting blood from rats injected intraperitoneally with 400 U.S.P. units of heparin 20 minutes before exsanguination. The blood thus obtained was centrifuged in plastic tubes at 2800 r.p.m. for 1 hour and then stored in a deep freeze unit for 24 hours. Pieces containing only packed erythrocytes and plasma, respectively, were broken off the two ends of the frozen column of centrifuged blood after its removal from the tube. These pieces were placed into other tubes, weighed, thawed out and counted in the gamma counter. For whole blood-serum radioiodide concentration ratio determinations the blood sample was obtained either by withdrawal into a heparinized syringe or by laking an aliquot of blood in distilled water. Serum was obtained from the same animals by severing the aorta, collecting the extravasated blood and centrifuging it in glass tubes. If heparinized blood was used, the radioactivity of known volumes of both blood and serum was determined by gamma-counting. If laked non-heparinized blood was utilized, measured blood and serum samples were subjected to beta counting. For plasma-serum radioiodide distribution studies plasma was obtained by centrifuging blood withdrawn with a heparinized syringe and serum by the centrifugation of blood collected from the abdominal cavity of the same animals after severance of the aorta. Beta counting of measured samples was used for radioactivity determinations. The amount of radioiodide absorbed from the lumen of the small intestine was computed by comparing the activity of the

intraintestinally injected tracer with that of the dissected small intestine after the half-hour *in vivo* sojourn of the tracer in its lumen. The gamma counter was used in this experiment.

5. *Radioiodide space determinations*

The formula used for the computation of the radioiodide space of various organs (expressed as % of their total volume) was based on the assumption that the radiohalogen is confined to the extracellular phase. This was done in order to facilitate comparison with similarly computed organ radioiodide and radiobromide spaces reported by others (Leblond, 1942, Perlman *et al.*, 1941).

When O/S ratios were determined, the radioiodide space of the organs was expressed as

$$\frac{\text{organ radioiodide concentration} \times 0.93 \times 0.95 \times 100}{\text{serum radioiodide concentration} \times 0.99},$$

where 0.93 and 0.99 are the correction factors for the solid contents of serum and tissue fluid, respectively, and 0.95 is the factor for the Gibbs-Donnan equilibrium. When the radioiodide concentration of whole blood was measured instead of that of serum (Table 1), the above formula was modified in accordance with the observed blood-serum radioiodide concentration ratios (Table 2) and the thyroid radioiodide space was therefore expressed as

$$\frac{\text{thyroid radioiodide concentration} \times 0.93 \times 0.95 \times 0.75 \times 100}{\text{blood radioiodide concentrations} \times 0.99}.$$

TABLE 2. DISTRIBUTION OF RADIOIODIDE IN BLOOD (1–1½ HOURS AFTER SUBCUTANEOUS INJECTION)

Radioiodide concentration ratio	$NaClO_4$ (100 mg.)	NaCl (47.5 mg.)	0
Erythrocytes/plasma	(10) 0.59 ± 0.016	(10) 0.59 ± 0.028	(9) 0.55 ± 0.013
Plasma/serum	(9) 0.95 ± 0.096	(10) 0.98 ± 0.976	—
Whole blood/serum			
a) Heparinized blood	(28) 0.75 ± 0.014	(29) 0.76 ± 0.013	(9) 0.78 ± 0.022
b) Non-heparinized blood	—	—	(20) 0.80 ± 0.010

Number of rats in parentheses. Means ± standard errors.

The body radioiodide space was defined as the volume in which radioiodide would be distributed if its concentration throughout the body were the same as in serum. In expressing this volume as % of body weight the difference between the densities of serum and whole body was neglected.

6. *Histological methods*

Thyroids were fixed in Susa, embedded in paraffin, cut at 6 μ and stained with periodic acid-Schiff-trichrome. The proportion of the various components of the gland was determined on projected slides by the linear measurement technique of Votila and Kallas (1952), using sections taken at three different levels from each gland.

7. *Statistical analysis of results*

Differences between the means of measurements obtained in comparable groups were analyzed with the aid of Student's "t" test and are called significant in the text if $P < 0.01$.

RESULTS AND DISCUSSION

1. *The radioiodide space of perchlorate-blocked thyroids*

The experiments summarized in Table 1 showed that the radioiodide space of perchlorate-blocked thyroids a) was approximately three times as great as the histologically determined stromal compartment of the gland; b) did not vary in size when alterations of the level of thyrotrophic stimulation caused enlargement of the thyroid or changes in the percentage of thyroid volume occupied by stroma plus parenchymal cells and, on the functional side, profound variations in the activity of the iodide "trap"; c) was not affected by the administration of a large dose of stable iodide before the injection of the tracer, and d) was as great 1–1½ hours after the injection of radioiodide as it was at 4–4½ hours.

Comment. Circumstantial evidence for the anatomical location of the iodide "trap" could only be expected from the determination of the radioiodide space of perchlorate-blocked thyroids (which iodide presumably enters by diffusion only), if the interface across which iodide is actively transported were impenetrable to diffusing iodide. If this premise were fulfilled, and a) the iodide pump were located along the thyroid cell-stromal boundary, the radioiodide space after perchlorate block should be no greater than the stromal compartment of the thyroid; b) if the site of the pump were the thyroid cell-colloid interface, the radioiodide space of the perchlorate-blocked gland should vary parallel with changes in the percentage of thyroid volume occupied by stroma plus parenchymal cells. Actually, the thyroidal radioiodide space after perchlorate treatment was three times as great as the stromal space. Such an extension of the radioiodide space beyond the stromal compartment could hardly have been stimulated by adsorption of radioiodide to structures contained in or bounding the stroma, since the magnitude of the space was not affected by a large dose of stable iodide. Stable iodide would have competed with radioiodide for loci of binding, and thereby would have diminished any apparent enlargement of the radioiodide space due to adsorption of the tracer. Further, the radioiodide space of perchlorate-blocked thyroids was remarkably constant in our experimental groups, although the magnitude of the thyroidal "stroma plus parenchymal cells" compartment was significantly enhanced by thyrotrophin (TSH) and diminished by its lack. Thus there was no indication that iodide diffuses up to but not across the thyroid cell-colloid interface, and there was evidence that the basal portion of the thyroid cell membrane is permeable to diffusing iodide. Our experiments could therefore provide no evidence for the anatomical site of the thyroidal iodide pump. As regards the second objective of our investigations, our results gave no indication that TSH promotes the diffusion of iodide into the thyroid parenchyma.

The constancy of the radioiodide space of perchlorate-blocked thyroids

under a variety of experimental conditions may indicate that in such glands iodide diffuses throughout the stroma and parenchyma, including the colloid. If such is the case, an explanation must be sought for the fact that the thyroidal radioiodide space is only a fraction of the space occupied by the total water of the gland. Thyroidal water content, as determined by desiccation, was found to average 78% of the gland's weight. So far as the thyroid is concerned, equilibration of radioiodide must have been complete by 1–1½ hours after the administration of the tracer, since no increase in the thyroid-blood radioiodide concentration ratio was observed when the rats were sacrificed 4–4½ hours after the injection. Two factors that could hamper the diffusion of radioiodide into the thyroid are a) the electric potential of the thyroid cell membrane and b) complexing of radioiodide with plasma proteins. We are not aware of any information concerning the properties of the thyroid cell membrane. The distribution of radioiodide among the components of blood was the subject of the second series of experiments included in this study.

2. *The distribution of radioiodide in blood (Table 2)*

The erythrocyte-plasma radioiodide concentration (RBC/Pl) ratio was lower than that observed by Boatman and Moses (1951) in rats and by Rall *et al.* (1950) in human blood *in vitro*. The plasma-serum radioiodide concentration ratio was probably not significantly different from 1. The whole blood-serum radioiodide concentration (B/S) ratio can be determined from the hematocrit (H) and the RBC/Pl ratio by the following formula:

$$B/S = \frac{RBC/Pl \times H + 100 - H}{100}.$$

In our experience the hematocrit values of rats (pooled data for animals which received 100 mg. of $NaClO_4$, 47.5 mg. of NaCl or no salt) ranged from 42 to 52 and averaged 48. With the hematocrit at 48 and the RBC/Pl ratio at 0.57, the computed B/S ratio is 0.79. This is in good agreement with the values which we obtained by actual determination of this ratio.

The distribution of radioiodide in blood did not appear to be significantly affected by treatment of the rats with 100 mg. of $NaClO_4$ or equimolecular amounts of NaCl, nor were the B/S ratios obtained by using *in vitro* heparinized blood different from those determined without the use of heparin.

Comment. The reliability of our direct measurement of the RBC/Pl ratio was supported by the agreement between the B/S ratio computed on its basis and that determined experimentally. If the erythrocyte water content is 65% and that of plasma 93% (Rall *et al.*, 1950), and if radioiodide distributed itself evenly throughout both plasma and erythrocyte water, without the occurrence of adsorption or binding, the RBC/Pl ratio should be 0.70. In our experiments it was only approximately 80% of this

value. This may have been due to a) electrolytic dissociation of hemoglobin or b) binding of radioiodide by plasma proteins. The lack or difference between plasma and serum radioiodide concentrations militates against a quantitatively important binding of radioiodide to fibrinogen. Complexing of radioiodide with other plasma proteins may occur and may be the only reason for the apparent disparity between the radioiodide space and the water space of the erythrocyte.[6] However, it cannot fully account for the much greater difference between these spaces in the perchlorate-blocked thyroid and in other organs which will be subsequently discussed.

3. *A survey of the radioiodide space of various organs*

We examined the organ-serum radioiodide concentration (O/S) ratio of a variety of organs and computed their radioiodide space on the basis of these measurements (Table 3). With the exception of the stomach wall

TABLE 3. ORGAN-SERUM RADIOIODIDE CONCENTRATION (O/S) RATIOS AND ORGAN RADIOIODIDE SPACES (AS % OF VOLUME) 1½ HOURS AFTER THE ADMINISTRATION OF I^{131}. EFFECT OF PERCHLORATE

Organ	Expt. No.	Treatment					
		47.5 mg. NaCl		0		100 mg. $NaClO_4$	
		O/S ratio	I^{131} Space	O/S ratio	I^{131} Space	O/S ratio	I^{131} Space
Kidney	II	(10) 0.66 ± 0.024	58%	—	—	(8) 0.70 ± 0.035	61%
Lung	III	(6) 0.60 ± 0.018	53%	—	—	(6) 0.62 ± 0.018	54%
Skin	III	(5) 0.53 ± 0.027	47%	—	—	(6) 0.54 ± 0.014	48%
Submaxillary gland	VI	(9) 0.41 ± 0.022	36%	—	—	(8) 0.42 ± 0.030	37%
Submaxillary gland	XVIII	—	—	(11) 0.39 ± 0.014	34%	—	—
Parotid	XVII	(6) 0.39 ± 0.026	34%	—	—	(6) 0.33 ± 0.023	29%
Spleen	III	(5) 0.38 ± 0.013	33%	—	—	(6) 0.34 ± 0.010	30%
Liver	II	(10) 0.38 ± 0.013	33%	—	—	(8) 0.36 ± 0.018	32%
Liver	XIX	—	—	(7) 0.34 ± 0.011	30%	—	—
Thyroid	I	—	—	—	—	(10) 0.37 ± 0.016	33%
Pituitary	VI	(9) 0.32 ± 0.014	28%	—	—	(10) 0.31 ± 0.012	27%
Pituitary	XIX	—	—	(11) 0.30 ± 0.008	26%	—	—
Adrenal	VI	(10) 0.29 ± 0.021	25%	—	—	(10) 0.29 ± 0.012	25%
Adrenal	XIX	—	—	(11) 0.29 ± 0.012	25%	—	—
Testis	III	(5) 0.22 ± 0.007	19%	—	—	(6) 0.23 ± 0.008	20%
Diaphragm	VI	(10) 0.20 ± 0.011	18%	—	—	(10) 0.20 ± 0.012	18%
Diaphragm	XIX	—	—	(11) 0.15 ± 0.008	13%	—	—

Number of rats per group in parentheses. Means ± standard errors. Experiments designated by the same Roman numeral were carried out simultaneously.

(Table 6), which will be discussed later, and, of course, the non-blocked thyroid (Table 1), the O/S ratios were less than 1 and were not significantly affected by 100 mg. of $NaClO_4$ if the determinations were made 1–1½ hours after the administration of the tracer. The O/S ratios of rats given no chloride were not significantly different from those of rats which received 47.5 mg. of NaCl, with the exception of the diaphragm-serum ratio, which was lower if the animals were not given salt. The radioiodide space of the perchlorate-blocked thyroid was lower than that of kidney, lung and skin, not reliably different from that of salivary glands, spleen

[6] Attempts to show whether some serum radioiodide was $ZnSO_4$-precipitable because of protein-binding were inconclusive, since addition of $Zn(OH)_2$-$BaSO_4$ to a solution of radioiodide in distilled water also resulted in absorption of varying amounts of radioiodide to the precipitate.

and liver, and higher than that of pituitary, adrenal, testis and skeletal muscle.

Comment. The O/S ratios listed in Table 3 do not differ markedly from those obtained for some of these organs by Wallace and Brodie (1937) with stable iodide in dogs and by Leblond (1942) with radioiodide in rats. Wallace and Brodie considered the iodide space to be coextensive with the extracellular space. Leblond, on the other hand, believed that radioiodide does enter cells.

Although we made no quantitative determinations of the stromal compartments of organs other than the thyroid, mere inspection of histologic preparations suggest that the radioiodide space is considerably greater than the extracellular space in all glands that were examined, with the possible exception of the testis. It would be hazardous to attempt a histologic estimation of the extracellular compartment in lung, spleen and skin. In the lung the extremely rich capillary network is in all probability largely responsible for the high O/S ratio. The skin used in our experiments was taken from the anterior aspect of the neck and included the hairs but not the subcutis. Although hair is known to concentrate iodide (Leblond, 1954), this process must be slow, since plucked hair showed negligible radioactivity 1–1½ hours after the administration of radioiodide. The comparatively high O/S ratio of skin samples which consisted of the dense fibrous dermis, the epidermis and its appendages suggests that radioiodide in this organ is not confined to the vascular and tissue fluid compartments, but may penetrate into collagenous fibers, as does chloride (Manery, 1954). The fact that the kidney showed a high O/S ratio is not surprising, since radioiodide-containing urine is concentrated in its nephra. The probable uptake of radioiodide by renal tubular cells will be discussed later. The radioiodide space of striated muscle is probably somewhat larger than its chloride space (cf. Woodbury, 1954). Penetration of some radioiodide into the muscle fibers therefore appears likely.

Of special interest is the fact that salivary glands showed no evidence of an iodide concentrating mechanism similar to that of the stomach (cf. Tables 3 and 6). The existence of concentrated iodide in human saliva as well as gastric juice is well established (Honour *et al.*, 1952). Freinkel and Ingbar (1953) have shown that the human salivary iodide concentrating mechanism is inhibited by thiocyanate. A considerable portion of the combined gastric-salivary radioiodide in rats is contributed by the saliva (Brown, 1955). It remains to be explained, therefore, why the rat's salivary glands failed to contain concentrated radioiodide under the conditions of our experiments, even though the gastric wall did. Johnson and Albert (1951) have also reported a radioiodide concentration of salivary gland (submaxillary according to a personal communication of Dr. Albert) lower than that of blood. The ineffectiveness of perchlorate on salivary gland-serum radioiodide concentration ratios is also in striking contrast with its

marked depressing effect on the gastric wall-serum ratio (Table 6). It is, of course, possible that the salivary iodide concentrating mechanism transports iodide across the apical boundary of the cells into the lumen, and that the cells themselves contain no concentrated iodide, but this may hold true for the gastric iodide pump as well. In the likely event of stagnation of saliva in the extensive duct system the iodide content of saliva should influence the salivary gland-serum iodide concentration ratio. Therefore, if a salivary iodide pump similar to that of the stomach existed in rats, one would expect a) a salivary gland-serum iodide concentration ratio higher than that observed and b) a marked influence of perchlorate on this ratio.

It is interesting to compare the radiobromide space (Perlman *et al.*, 1941) of various organs with their radioiodide space. The radiobromide space of rat muscle was found to be 18%, which agrees with the radioiodide

TABLE 4. EFFECT OF PERCHLORATE ON THE BODY RADIOIODIDE SPACE OF NEPHRECTOMIZED RATS

Hours after nephrectomy	Hours after I^{131}	Radioiodide space, % of body weight	
		100 mg. $NaClO_4$	47.5 mg. NaCl
2–2½	1–1½	(5) 25.0 ± 0.9	(6) 34.5 ± 2.0
5–5½	1–1½	—	(3) 38.3 ± 1.3
5–5½	4–4½	(6) 36.2 ± 0.4	(5) 74.1 ± 4.7

Number of rats per group in parentheses. Means ± standard error.

space, but that of the liver was only 22% and that of the adrenal as high as 36%. Such discrepancies between the radioiodide and radiobromide spaces are not easily explained if one assumes that both radiohalogens are confined to the extracellular phase of these organs.

In conclusion, radioiodide appears to diffuse into the cells of at least some organs beside the thyroid. The radioiodide space of all organs examined (with the exception of the thyroid and the stomach wall of animals that had not received perchlorate) appeared to be less than their total water space. The electric properties of the plasma membranes of different cells may play a role in determining the characteristic magnitude of the radioiodide space of different organs.

4. *Effect of perchlorate on the body radioiodide space and the gastric iodide concentrating mechanism*

Having investigated the radioiodide space of various organs, we became interested in the radioiodide space of the whole body, and the effects of perchlorate thereupon. Table 4 shows that perchlorate significantly depressed the body radioiodide space of nephrectomized rats. In both the perchlorate-treated rats and the chloride-treated controls the magnitude of the body radioiodide space increased between 1 and 4 hours after the

administration of the tracer. However, this expansion of the space was much more marked in the chloride-treated animals.

In attempting to explain these observations, we compared organ-serum radioiodide concentration ratios determined 1–1½ hours and 4–4½ hours after the injection of I^{131}. Special attention was paid to organs which are large enough to influence the body iodide space, viz. liver, muscles and skin. Both nephrectomized and unoperated rats were used (Table 5). Neither the difference between the body radioiodide space of perchlorate-treated and chloride-treated rats nor the expansion of this space with time was explained by the findings summarized in Table 5. The organ-serum radioiodide concentration ratios for muscle, skin, submaxillary gland and liver may have been slightly higher 4–4½ hours than 1–1½ hours after the injection of I^{131} in the chloride-treated group, but no such difference was

TABLE 5. ORGAN-SERUM RADIOIODIDE CONCENTRATION (O/S) RATIOS 1–1½, 4–4½ AND 24–24½ HOURS AFTER ADMINISTRATION OF THE TRACER. EFFECT OF PERCHLORATE OR STABLE IODIDE

Organ	Dose of PTU, mg.	Expt. no.	Hours after nephrectomy	Hours after I^{131}	O/S ratio		
					47.5 mg. NaCl	100 mg. $NaClO_4$	122 mg. NaI
Skeletal muscle:							
diaphragm	6	VI	—	1– 1½	(10) 0.20±0.011	(10) 0.20±0.012	—
gastrocnemius	6	VIII	—	4– 4½	(6) 0.23±0.021	(6) 0.19±0.020	—
gastrocnemius	6	IX	—	4– 4½	(9) 0.23±0.013	—	(9) 0.19±0.008
gastrocnemius	6	V	5–5½	4– 4½	(6) 0.18±0.006	(6) 0.20±0.009	—
Skin	6	III	—	1– 1½	(5) 0.53±0.027	(6) 0.54±0.014	—
Skin	6	VIII	—	4– 4½	(6) 0.65±0.046	(6) 0.56±0.028	—
Skin	6	V	5–5½	4– 4½	(6) 0.59±0.016	(6) 0.53±0.012	—
Submaxillary gland	6	VI	—	1– 1½	(9) 0.41±0.022	(8) 0.42±0.030	—
Submaxillary gland	6	VII	—	4– 4½	(5) 0.48±0.040	(7) 0.39±0.032	—
Adrenal	6	VI	—	1– 1½	(10) 0.29±0.021	(10) 0.29±0.012	—
Adrenal	6	VII	—	4– 4½	(7) 0.29±0.021	(7) 0.26±0.010	—
Liver	6	II	—	1– 1½	(10) 0.38±0.013	(8) 0.36±0.018	—
Liver	6	VIII	—	4– 4½	(6) 0.42±0.018	(6) 0.34±0.015	—
Liver	6	V	5–5½	4– 4½	(6) 0.37±0.018	(6) 0.32±0.006	—
Liver	6	IX	—	4– 4½	(10) 0.47±0.022	—	(10) 0.34±0.018
Liver	52	XVI	—	24–24½	(8) 0.44±0.020	(8) 0.33±0.031	—
Liver	52	XVI	—	1– 1½	(8) 0.32±0.010	—	—

Number of rats per group in parentheses. Means ± standard errors. Experiments designated by the same Roman numeral were carried out simultaneously.

observed in the perchlorate-treated group. Further, any depressing effect that perchlorate may have had on organ-serum radioiodide concentration ratios was minimal in comparison with its influence on the radioiodide space of the whole body.

Subsequent investigations showed that the effect of perchlorate on body radioiodide space is probably largely due to inhibition of the gastric iodide concentrating mechanism. Table 6 shows that the gastric wall-serum radioiodide gradient, which was somewhat greater than 1 and did not increase with time, was greatly depressed by perchlorate. More significantly, the gastric juice-serum radioiodide concentration ratio, which was above 15, was reduced to less than 1 by perchlorate. The gastric juice-serum radioiodide gradient was lower in nephrectomized rats and was markedly greater in such animals when determined 4–4½ hours after I^{131} injection than at 1–1½ hours.

Comment. The shape of the time curve of the body radioiodide space in chloride-treated nephrectomized rats suggests that equilibration of the radioisotope may not have been complete 4 hours after its injection. Myant *et al.* (1950) have also found that the radioiodide space of the human body expands up to 5 hours after administration of the tracer. The body radioiodide space of man, however, was substantially lower (approximately 36% of body weight) than that of rats after 4 hours of equilibration of I^{131}. The body stable iodide space of dogs (Wallace and Brodie, 1937) was also in the vicinity of 40% of body weight. Our findings regarding the large size of the body radioiodide space of nephrectomized rats are, however, in good agreement with Ingbar's (1953) estimate of this space in intact rats. He found that 2–8 hours after the administration of a tracer dose of radioiodide it was distributed in a volume equaling that of the entire body.

Since we could confirm earlier observations (Leblond, 1942 and others) regarding the concentration of radioiodide in the stomach wall and, especially, in the gastric contents, we concluded that the gastric stores of concentrated radioiodide must greatly expand the body radioiodide space

TABLE 6. EFFECT OF PERCHLORATE ON THE STOMACH WALL-SERUM AND GASTRIC JUICE-SERUM RADIOIODIDE CONCENTRATION RATIOS

	Expt. no.	Hours after nephrectomy	Hours after I^{131}	Treatment	
				47.5 mg. NaCl	100 mg. $NaClO_4$
Stomach wall/serum	II	—	1–1½	(10) 1.45±0.10	(8) 0.36±0.020
Stomach wall/serum	XIII	—	4–4½	(5) 1.44±0.26	—
Gastric juice/serum	XIV	—	4–4½	(8) 15.8±2.0	(7) 0.75±0.050
Gastric juice/serum	XV	5–5½	1–1½	(5) 1.9±0.4	—
Gastric juice/serum	XV	5–5½	4–4½	(3) 5.8±0.8	—

Number of rats per group in parentheses. Means ± standard errors.

Inhibition of the gastric iodide concentrating mechanism was previously achieved by means of thiocyanate (Mason, 1952). Perchlorate was now shown to have the same effect. The reduction of the body radioiodide space after perchlorate treatment was therefore easily explained.

The observed increase with time in the body radioiodide space of chloride-treated nephrectomized rats became understandable when we demonstrated that the gastric juice-serum radioiodide gradient increased *pari passu* in such animals (Table 6).

The observation that the gastric juice-serum radioiodide concentration ratio was greater in intact than in nephrectomized rats (Table 6) may be tentatively interpreted by assuming that the kinetics of gastric radioiodide concentrating are essentially similar to those of thyroidal radioiodide "trapping." Wollman (1954) has pointed out that the thyroid-serum radioiodide gradient will rise if the rate of excretion of radioiodide is great relative to that of its equilibration. Gastric radioiodide concentration seems to have a rate of equilibration which is slower than that of thyroidal radioiodide "trapping." Hence, if the gastric and thyroidal iodide pumps are

comparable, stomach juice-serum radioiodide gradients should be even more readily influenced by changes in the rate of radioiodide excretion than are T/S ratios. One would therefore expect a markedly higher gastric juice-serum radioiodide gradient in animals with intact kidneys than in nephrectomized animals.

The body radioiodide space of perchlorate-treated nephrectomized rats (whose gastric iodide pump was presumably nonfunctional) significantly increased with time. This could not be ascribed to an expansion of the radioiodide space of any of the organs examined. It is possible that there was a slow diffusion of radioiodide into some organ system that was not investigated, e.g. the skeleton and the central nervous system. The body radioiodide space of 36% which was found 4–4½ hours after the injection of I^{131} in perchlorate-treated rats exceeds the extracellular space estimated in other species by any of the conventional methods (Elkington and Danowski, 1955). This is in agreement with the suggestion that radioiodide is not confined to the extracellular phase, even when the iodide pumps of the body are blocked.

5. *Effect of perchlorate and stable iodide on hepatic radioiodide uptake*

Leblond (1942) found less radioiodide in the livers of rats after pretreatment with large doses of stable iodide than if no stable iodide was administered. He suggested that the liver possesses a mechanism for the accumulation of iodine which can be saturated. Our findings confirmed and extended these observations (Table 5). The liver-serum radioiodide concentration (L/S) ratio was not significantly altered by perchlorate-pretreatment if the determination was made 1–1½ hours after the injection of I^{131}. Four to 4½ hours after the administration of the tracer, however, the L/S ratio was significantly lower in perchlorate-treated rats than in chloride-treated controls. Stable iodide in amounts equimolecular to those of perchlorate had the same effect. The L/S ratio had not increased beyond the 4–4½ hour value when it was determined 24–24½ hours after radioiodide administration (1–1½ hours after the injection of NaCl). The 24–24½ hour L/S ratio was significantly lowered when $NaClO_4$ was injected instead of NaCl 1–1½ hours before the rats were killed. The rats used for the determination of the 24–24½ hour L/S ratio had received 52 mg. of propylthiouracil (PTU) instead of the customary 6 mg. In order to examine the possible effect of this pretreatment *per se* on hepatic radioiodide uptake, the 1–1½ hour L/S ratio was also examined in such animals. It was significantly lower than the 24–24½ hour ratio of rats which had not received perchlorate and was not reliably different from the L/S ratios found at any time interval after I^{131} administration in perchlorate-treated rats.

Comment. Our findings indicate that not all of the hepatic radioiodide enters the organ by simple diffusion. Rather, there appears to exist another hepatic iodide space in addition to the iodide diffusion space of the organ.

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The entry of radioiodide into this second compartment occurs slowly and can be inhibited with stable iodide as well as perchlorate. Furthermore, radioiodide can be discharged from this compartment by means of perchlorate. Lastly, once radioiodide has entered the compartment, it seems to equilibrate with serum iodide, and no further increase in the L/S ratio occurs with time. Such an increase would be expected if radioiodide became bound within the liver instead of remaining freely exchangeable. Our results are compatible with the idea that the liver possesses an iodide pump essentially comparable with that of the thyroid, except that the equilibration of pumped hepatic iodide with serum iodide is slower than in the case of the thyroid, and that the hepatic mechanism is probably much less efficient than its thyroidal counterpart, since the thyroid-serum radioiodide gradient may reach 400, whereas the liver-serum radioiodide concentration does not exceed 0.5.

6. *Effect of perchlorate on the intestinal absorption and the excretion of radioiodide*

Table 6 shows that even after the administration of a dose of perchlorate which presumably blocked the gastric iodide pump as completely as it blocked that of the thyroid, radioiodide entered the gastric juice. Since the epithelium of the stomach is interposed between the blood vessels of the stomach wall and the gastric lumen, diffusing radioiodide must have passed through the epithelial cells or the intercellular cement substance. A similar passage of iodide across the intestinal epithelium must occur, since little of the gastric radioiodide can be recovered in the feces (Leblond, 1942). We found that intestinal absorption of radioiodide is not specifically affected by perchlorate and may therefore be due to simple diffusion. In 4 rats which were injected with perchlorate the mean absorption of a dose of radioiodide from the small intestine was 78% within one-half hour. In 3 rats injected with chloride instead of perchlorate an average of 64% of an intraintestinally injected dose of radioiodide was absorbed during the same period.

We have reasons to believe that iodide enters the cells of the renal tubules. The fact that a) renal clearance of iodide is promoted by chloride (Riggs, 1949) and that b) iodide deficient diets are more goitrogenic if they are supplemented with NaCl (Axelrad *et al.*, 1955) is most easily explained by the assumption that chloride competes with iodide for a renal tubular absorptive mechanism. Our experiments suggest that perchlorate is even more effective in this regard. The 4 hour urinary excretion of radioiodide in the study of Johnson and Albert (1951) on rats which received a commercial regimen amounted to approximately 16% of the administered dose. In 6 of our rats which were injected with 47.5% mg. of NaCl the 4 hour loss of radioiodide from the body was $31 \pm 3\%$ (mean $\pm$ standard error) of the dose. This value was arrived at by comparing the activity of

the injected tracer with that of blood samples and taking into account the magnitude of the body radioiodide space as determined in nephrectomized rats (Table 4). Similar measurements and calculations indicated that in 6 rats treated with 100 mg. of $NaClO_4$ $62 \pm 1\%$ of an injected radioiodide tracer was excreted within 4 hours. In view of these results a systematic study of the effect of various anions on the renal clearance of radioiodide is called for.

CONCLUSIONS

The following is a brief synopsis of the distribution of radioiodide in the body of propylthiouracil-treated rats and the effects of perchlorate thereupon.

The thyroid and the stomach possess iodide pumps which can be inhibited by perchlorate. Although iodide concentrations many times those of serum can be found in the thyroid and in gastric juice, the stomach wall-serum iodide concentration ratio is not much over 1. There is no cogent evidence that iodide is concentrated within any cell of the mammalian body. The thyroidal iodide concentrating mechanism may transport iodide across the apical membrane of the thyroid cell into the colloid. The presence of concentrated radioiodide in the follicular lumen has been radioautographically demonstrated by Pitt-Rivers and Trotter (1953) and Doniach and Logothetopoulos (1955). The stagnation of some gastric juice with its highly concentrated iodide in the canaliculi and glandular lumina of the stomach mucosa could fully explain why the iodide concentration of the stomach wall exceeds that of serum.

Curiously, there seems to be no evidence for the existence of a salivary iodide pump in the rat. On the other hand, the liver appears to possess a mechanism for the uptake of iodide which is probably less efficient than that of the thyroid or even the gastric wall but nevertheless may be essentially similar, since it too is inhibited by perchlorate. This hepatic iodide pump (?) may also be concerned with the transfer of iodide into the bile rather than into an intracellular compartment.

There is circumstantial evidence for the existence of a renal tubular mechanism concerned with the active uptake of iodide from the glomerular filtrate. This mechanism seems to be blocked by perchlorate and, although less efficiently, also by chloride, which is not known to affect the iodide pumps of thyroid and stomach.

When the iodide concentrating mechanisms of the body are blocked with perchlorate, iodide probably diffuses through the epithelial lining of the stomach. It is absorbed from the small intestine, presumably also by diffusion through the epithelium. It enters the erythrocyte. The iodide space of several organs, including the perchlorate-blocked thyroid, is clearly larger than their stromal compartment. This suggests that iodide diffuses into a wide variety of cells. In fact, there is no conclusive evidence that iodide remains confined to the extracellular compartment of any organ-

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SUMMARY

1. The radioiodide space of rat thyroids, the iodide "trap" of which was completely blocked with sodium perchlorate, was about 30% of the total gland volume, whereas the histologically determined stromal compartment was approximately 10%. This suggests that radioiodide can diffuse into thyroid cells. Thyrotrophin had no effect on the thyroidal radioiodide diffusion space.

2. The radioiodide space of erythrocytes was approximately 80% of their water space. The radioiodide concentration of plasma was not significantly different from that of serum. Perchlorate did not affect the distribution of radioiodide among the components of blood.

3. The radioiodide space of kidney, lung and skin was greater than that of the perchlorate-blocked thyroid, that of salivary glands, spleen and liver about the same, and that of pituitary, adrenal, testis and skeletal muscle smaller. The radioiodide space of these organs (determined 1–1½ hours after the administration of the tracer) was not significantly affected by perchlorate. It is suggested that radioiodide diffuses into the cells of at least some organs other than the thyroid.

4. The body radioiodide space of nephrectomized rats treated with perchlorate increased from 24% of the body weight to 36% between 1 and 4 hours after the administration of I^{131}. In chloride-treated controls the corresponding values were 35% and 74%. The diminution of the radioiodide space in perchlorate-treated animals is believed to be largely due to inhibition of the gastric iodide concentrating mechanism.

5. The liver appears to possess an iodide compartment which equilibrates slowly with serum iodide. The entry of radioiodide into this compartment was prevented by perchlorate or stable iodide. Perchlorate discharged radioiodide from the compartment.

6. Intestinal absorption of radioiodide was at least as fast in perchlorate-treated rats as in chloride-treated controls. Perchlorate appeared more effective than chloride in enhancing the excretion of radioiodide, presumably by interfering with its renal tubular absorption.

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THE EFFECT OF THYROID STIMULATING HORMONE UPON THE IODIDE COLLECTING MECHANISM OF THYROID TISSUE SLICES[1]

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IN SPITE of an increasing number of techniques for the assay of thyroid stimulating hormone (TSH), none of the methods has been generally adopted. The reported assays continue to be limited in usefulness because of their complexity, variability, and limited sensitivity. Sensitivity is most important since it is desirable to measure directly and without an intervening concentration procedure the small amounts of the hormone present in body fluids. The present communication describes conditions for studying radioiodide uptake and release from beef thyroid slices as these processes were altered by the *in vitro* addition of TSH. It is hoped that these experiments will provide a basis for the development of an assay for TSH.

Tissue slice experiments have several advantages over *in vivo* systems for detecting TSH: (1) the added TSH can be confined in a small volume with the target tissue, thus avoiding dilution throughout the body as occurs in *in vivo* experiments; (2) only the isolated target tissue is present, avoiding catabolism of TSH in other tissues which may normally account for the breakdown of most of the circulating TSH (D'Angelo, 1955); (3) a large number (up to 65 in this study) of comparable slices can be prepared rapidly from a single lobe of beef thyroid and incubated together in the same incubator permitting the convenient accumulation of sufficient replicate data to validate small differences between control and experimental groups.

Surviving thyroid slices preserve many aspects of iodine metabolism found *in vivo* including the collection and concentration of inorganic iodide (Morton and Chaikoff, 1943) followed by the organic binding of this ion

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